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Toxicology Branch I - IRS (H7509C) 10/23/90

DATA EVALUATION RECORD 008139

I. SUMMARY

MRID (Acc.) No.: 412552-22
ID No.: 7078-RT
RD Record No.: 253,112
Caswell No.: 623C (129017)
Project No.: 0-0339

Study Type: Mutagenicity - Gene mutation in bacteria (Ames Assay)

Chemical: CIDEX OPA Antimicrobial (ortho-phthalaldehyde)

Sponsor: Surgikos, Inc., Arlington, TX

Testing Facility: Microbiological Associates (M/A) Bethesda, MD

Title of Report: Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test).

Authors: T.E. Lawlor and V.O. Wagner

Study Number: (M/A)T5203.501027

Date of Issue: November 25, 1986

TB Conclusions:

Negative for inducing reversions in the Ames battery of Salmonella TA strains (his$^-$ to his$^+$) exposed with/without rat S9 activation up to cytotoxic levels (20 ug/plate -S9; 60 to 90 ug/plate +S9).

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - Ortho-phthalaldehyde (OPA) (KC839-84)

Description: Crystalline solid
Batch (Lot): Eastman Kodak E15
Purity (%): 99
Solvent/Carrier/Diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Bacterial strains

Species: Salmonella typhimurium LT2
Strain: TA98, TA100, TA1535, TA1537, TA1538, TA102, and TA104 (all his$^-$)
Source: Dr. Bruce Ames, UCal (Berkeley)

C. Study Design (Protocol) - This study was designed to assess the mutagenic potential of CIDEX technical when administered in vitro to bacterial cultures (Salmonella his$^-$ strains) according to a protocol (provided as appended Section G of the Final Report) based upon the published (validated) procedures of Ames and associates.

A Statement of Quality Assurance measures (inspections/audits) was provided, as well as a statement of adherence to Good Laboratory Practice.

D. Procedures/Methods of Analysis - Following dose range-finding tests with one of the TA strains (TA100 treated with 10 concentrations of OPA ranging from 10 to 10,000 ug/plate), triplicate cultures of all seven TA strains were exposed to five dose levels of test article, both in the absence and presence of a mammalian metabolic activation system, consisting of the microsomal (S9) fraction of liver homogenates prepared from male Sprague-Dawley (SD) rats pretreated with Aroclor 1254, plus NADP(H)-generating cofactors. In addition to DMSO (negative solvent) controls, other cultures were exposed to strain-specific mutagens*, to serve as positive controls.

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*Without Activation (-S9): TA98/TA1538 - 2-nitrofluorene (NF, 5 ug)
TA100/TA1535 - sodium azide (SA, 5 ug)
TA1537 - 9-aminoacridine (AA, 75 ug)
TA100 - cumene hydroperoxide (CH, 75 ug)
TA104 - methylmethanesulfonate (MMS, 500 ug)

With Activation (+S9): TA98/TA100/TA1535/TA1537/TA1538 - 2-amino-anthracene (Anth, 4 ug)
TA102/TA104 - sterigmacystin (SC, 10 ug)

After 48 hours incubation with test article, reverent colonies (his$^+$) were counted, either entirely by automated colony counter or by hand (the latter when sufficient precipitate interfered with automated counting). Criteria used by this lab for both determination of a valid test, as well as evaluation of test results, were presented.

E. Results - The results of the preliminary range-finding test indicated that the appropriate maximal dose level for the main assay would be 60 ug/plate with activation, and 20 ug/plate without, based upon evidence of cytotoxicity, such as severe reductions in revertent colonies and background lawn (Report Table 1). Four additional lower concentrations were also selected for the initial experiments of the main assay, namely: 0.4, 2, 10, and 30 ug/plate for activated trials (+S9); 0.08, 0.4, 2, and 10 ug/plate for nonactivated trials.

A total of four experiments were conducted, with individual plate data presented in Report Tables 2 through 19, and summarized in Report Tables 20 through 23 (appended to this DER).

In the initial trial, no significant increases in revertents (i.e., at least a doubling of solvent values) were found at test dose-treated TA100, TA1535, TA1537, and TA1538 either with or without S9, and cytotoxicity was evident at the HDTs (Table 20 attached). Whereas the negative results for nonactivated TA102 were acceptable, data from TA98 (±S9) could not be interpreted because of abnormal colony characteristics; those from TA104 (±S9) as well as activated (+S9) TA102 were also unacceptable because of control colony counts outside historical background.

Hence TA98, TA104, and TA102 were retested at the same doses. This trial revealed no increases in revertents for the first two strains with/without activation up to cytotoxic HDTs (Report Table 21, attached). However, activated TA102 cultures (+S9) did not grow properly and were not plated. In further testing of activated TA102, there were no increased colony counts, but also no toxicity at 60 ug/plate, the HDT (Report Table 22), which necessitated a fourth round of testing TA102/+S9 at higher doses (90 and 120 ug/plate). This trial had to be aborted because of poor growth of the cultures, but in the final experiment, an acceptable level of toxicity was achieved at the higher concentrations, but no increase in revertents (Report Table 23, attached).

The authors concluded that the test article, OPA technical, was negative for mutagenicity in Ames testing up to moderately severe levels of cytotoxicity.

F. TB Evaluation - ACCEPTABLE. The authors have demonstrated in repeat experiments by adequate procedures and controls that the test article, orthophthalaldehyde, did not induce reverse gene mutations in the Ames battery of Salmonella his$^-$ strains, tested up to levels of cytotoxicity with/without metabolic activation (rat S9).

Attachments (Report Summary Tables)

ATTACHMENT I

Summary Data Tables

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